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Performance Evaluation of the ADVIA Centaur Androstenedione Assay*

Parker L,[1] Driscoll J,[1] Higgins C,[1] Stranz M,[1] Garvey K,[1] Donovan P,[2] Zhao Z,[1] Zheng YF,[1] Freeman J.[1]
[1]Siemens Healthcare Diagnostics Inc., Newark, DE, U.S. [2]Siemens Healthcare Diagnostics Inc., Walpole, MA, U.S.

Abstract

Background: Androstenedione is a 19-carbon steroid that serves as a precursor for testosterone and estrone. It is primarily synthesized from dehydroepiandrosterone (DHEA) via 3ß-hydroxysteroid dehydrogenase in the ovaries, testes, and adrenal glands.[1-3]

Androstenedione is most commonly used in conjunction with other steroid assays to evaluate the function of the adrenal glands and ovaries or testes and to determine the cause of symptoms of androgen excess.[3-7] It is also used in the monitoring of treatment for congenital adrenal hyperplasia.[8,9]

A new ADVIA Centaur® Androstenedione (ANDRO) assay for the measurement of androstenedione in human serum and plasma has been evaluated by Siemens Healthineers. The studies below describe performance of the assay on the ADVIA Centaur XP Immunoassay System.

Methods: The ADVIA Centaur ANDRO assay is a fully automated competitive immunoassay using direct chemiluminescent technology. Reagents include a sheep monoclonal antibody coupled to paramagnetic particles in the solid phase and a novel acridinium ester in the Lite reagent. Solid phase and Lite reagent are incubated with 20 µL of patient sample. Competition for solid phase binding occurs between androstenedione in the sample and the Lite reagent. Separation follows, and the amount of signal generated is inversely proportional to the concentration of androstenedione in the sample. The time to first result is 18 minutes.

Results: The ADVIA Centaur ANDRO assay correlated well to LC-MS/MS across the measuring interval of 0.30 to 9.00 ng/mL. Equivalent performance was determined for serum, lithium heparin plasma, and potassium EDTA plasma sample types. Within-lab precision was <8% CV (with 95% confidence), and the assay demonstrated good specificity, with ≤10% interference and ≤1% cross-reactivity for the majority of the compounds evaluated. Stability data demonstrated a calibration interval and onboard stability of 35 days.

Conclusions: The ADVIA Centaur ANDRO assay demonstrates good precision, specificity, and correlation to LC-MS/MS.

Background

Hyperandrogenism can result from various disease states, including polycystic ovarian syndrome (PCOS), premature adrenarche, and congenital adrenal hyperplasia (CAH). The symptoms of androgen excess often include those of virilization, such as acne, hair loss, deepening of voice, and hirsutism. Androgen determination is used to differentiate benign conditions such as precocious puberty from those that are more serious, such as gonadal or adrenal tumors. It is also used to monitor treatment for patients with androgen excess, such as those with diagnosed CAH.[5,10-12]

The ADVIA Centaur Androstenedione (ANDRO) assay uses direct chemiluminescent technology to measure the concentration of androstenedione in human serum and plasma.

Methods

Principle of the assay

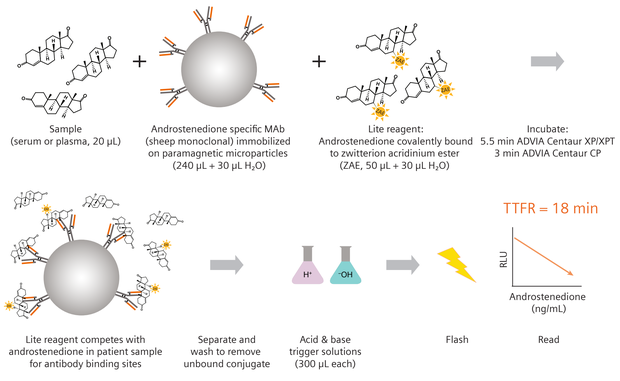

Figure 1. ADVIA Centaur ANDRO assay format.

Method comparison

A total of 129 native serum samples were evaluated using the ANDRO assay on the ADVIA Centaur XP Immunoassay system and LC-MS/MS (ARUP Laboratories, Salt Lake City, Utah; Quest Diagnostics, San Juan Capistrano, CA). Single-replicate data were plotted, and slope and intercept were determined using Passing-Bablok regression. The correlation coefficient (r) was determined using least squares regression.

Specimen equivalence

A total of 63 native and spiked matched serum, EDTA, and lithium heparin plasma samples were evaluated using the ANDRO assay on the ADVIA Centaur XP system. Single-replicate data were plotted, and slope and intercept were determined using Passing-Bablok regression. Correlation coefficients (r) were determined using least squares regression.

Precision

Bio-Rad LIQUICHEK Immunoassay Plus controls, calibrators, and patient samples distributed across the assay range were processed in replicates of two, twice a day, for 20 days using the ANDRO assay on the ADVIA Centaur XP system. Mean analyte values, repeatability, and within-lab precision were calculated for each sample, and 95% upper confidence limits were determined.

Interference

Serum samples spiked with potential interferents and matched controls were processed in replicates of three using the ANDRO assay on the ADVIA Centaur XP system. Interference was calculated as the % difference between the mean test and control sample results, with respect to the mean control sample result:

$$\% \text{ Interference} = 100 \times (\text{Mean}_{\text{Test}} - \text{Mean}_{\text{Control}})/(\text{Mean}_{\text{Control}})$$

Biotin interference was determined by titrating increasing amounts of biotin into serum samples and plotting ANDRO recovery versus biotin concentration.

Cross-reactivity

Serum samples spiked with potential cross-reactants and matched controls were processed in replicates of three using the ANDRO assay on the ADVIA Centaur XP system. Cross-reactivity was calculated as the % difference between the mean test and control sample results, with respect to the test compound concentration:

$$\% \text{ Cross-reactivity} = 100 \times (\text{Mean}_{\text{Test}} - \text{Mean}_{\text{Control}})/(\text{Concentration}_{\text{Test}})$$

Calibration interval and onboard stability

Bio-Rad LIQUICHEK Immunoassay Plus controls, calibrators, and patient samples distributed across the measuring interval were processed using the ANDRO assay on the ADVIA Centaur XP system in minimum replicates of four over 12 test days (study duration, 36 days). A fresh reagent pack was loaded at each time point, and mean observed analyte results were calculated.

Calibration interval was determined by performing a linear regression of the recoveries (Y-axis) versus test day (X-axis). A linear regression of normalized onboard means (Y-axis) versus test day (X-axis) was then plotted. If the regression slope was statistically significant ($p < 0.05$), the calibration interval for that sample was taken as the time at which the two-sided 90% confidence interval of the regression line intersected with the allowable drift-acceptance criterion. If the regression slope was not significant ($p \geq 0.05$), the calibration interval was taken as the study duration minus one day.

Onboard stability was performed such that >80% of the reagent pack was consumed over the duration of the study. Data was analyzed by plotting and performing a linear regression of the delta values (Y-axis) versus test day (X-axis). If the regression slope was statistically significant ($p < 0.05$), onboard stability for that sample was taken as the time at which the two-sided 90% confidence interval of the regression line intersected with the allowable drift-acceptance criterion. If the regression slope was not significant ($p \geq 0.05$), onboard stability was taken as the study duration minus one day.

Results

Method comparison

Evaluation of 129 samples using the ANDRO assay on the ADVIA Centaur XP system and LC-MS/MS Androstenedione demonstrated a slope of 1.06, intercept of –0.08 ng/mL, and r of 0.966.

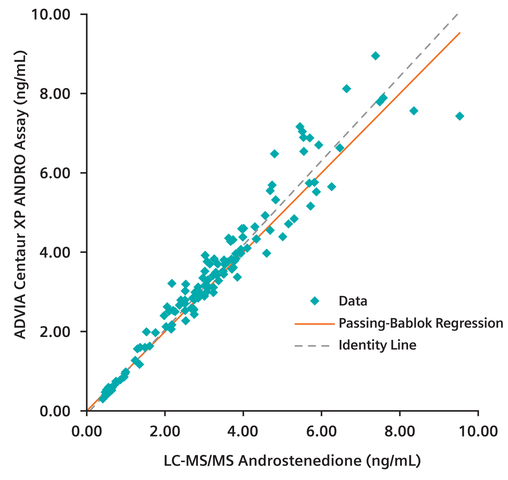

Figure 2. Method comparison of the ADVIA Centaur ANDRO assay versus LC-MS/MS Androstenedione via Passing-Bablok regression.

Specimen equivalence

Evaluation of 63 matched serum, EDTA plasma, and lithium heparin plasma samples using the ANDRO assay on the ADVIA Centaur XP system demonstrated similar performance of lithium heparin and EDTA plasma matrices versus serum with slope, intercept, and r values as shown in Table 1.

Table 1. Evaluation of specimen equivalence for the ADVIA Centaur ANDRO assay.

Sample Types	n	Slope	Y-intercept (ng/mL)	r
Lithium heparin plasma vs. serum	63	1.00	0.01	0.998
EDTA plasma vs. serum	63	1.02	0.10	0.998

Precision

Precision evaluation via 20-day ANOVA using the ANDRO assay on the ADVIA Centaur XP system demonstrated maximum repeatability and within-lab %CVs (with 95% confidence) of 5.0% and 7.2%, respectively.

Table 2. Evaluation of precision for the ADVIA Centaur ANDRO assay.

Sample ID	Grand Mean (ng/mL)	Repeatability			Within-Lab		
		SD	CV (%)	95% UCL[a]	SD	CV (%)	95% UCL[a]
Low Calibrator	0.69	0.025	3.6	4.4	0.036	5.3	6.3
High Calibrator	5.36	0.152	2.8	3.5	0.264	4.9	6.0
QC Level 2	1.07	0.031	2.9	3.6	0.043	4.0	4.8
QC Level 3	2.02	0.057	2.8	3.5	0.079	3.9	4.7
Serum 1	0.54	0.018	3.4	4.1	0.028	5.2	6.2
Serum 2	1.07	0.029	2.7	3.3	0.042	3.9	4.7
Serum 3	2.65	0.066	2.5	3.1	0.104	3.9	4.6
Serum 4	5.62	0.218	3.9	4.8	0.298	5.3	6.2
Lithium Heparin Plasma	0.45	0.018	4.1	5.0	0.027	6.1	7.2

a. UCL: upper confidence limit.

Interference

Ten of the 11 potential endogenous and exogenous interferents evaluated demonstrated ≤10% interference in the ANDRO assay on the ADVIA Centaur XP system. Biotin interference was ≤10% at biotin concentrations ≤635 ng/mL.

Table 3. Evaluation of interference for the ADVIA Centaur ANDRO assay.

Compound	Concentration Tested	% Interference	
		0.80–1.20 ng/mL ANDRO	2.00–3.00 ng/mL ANDRO
Hemoglobin	2 g/L	-0.9	0.3
Lipemia (INTRALIPID)	1000 mg/dL	-1.2	-3.0
Bilirubin (conjugated)	0.2 g/L	-0.4	-1.0
Bilirubin (unconjugated)	0.2 g/L	1.2	2.3
Silwet L720	0.03 mg/mL	-3.0	-2.7
Cholesterol	5 g/L	4.2	-3.0
Human gamma globulin	60 g/L	0.0	2.7
Human serum albumin	60 g/L	6.0	-2.2
Total protein	120 g/L	2.0	-6.1
Rheumatoid factor	1000 IU/mL	9.2	-2.6

Cross-reactivity

Over 40 endogenous and exogenous potential cross-reactants were evaluated using the ANDRO assay on the ADVIA Centaur XP system. Of these, only the aromatase inhibitors exemestane and formestane demonstrated >1% cross-reactivity. The potential cross-reactants evaluated included:

Adrenosterone
Aldosterone
5ß-Androstan-3α, 17ß-diol
Δ4-Androsten-11ß-ol-3, 17-dione
Androsterone
Canrenone
Cholesterol
Corticosterone
Cortisol
Cortisone
Deoxycorticosterone
Dexamethasone
DHEA
DHEA-SO_4
5α-Dihydrotestosterone
Dutasteride
Epitestosterone
Estradiol-17ß
Estriol
Estrone
Etiocholanone
Exemestane
Finasteride
Fludrocortisone acetate
Fluticasone propionate
Formestane
Fulvestrant
Glycyrrhetic acid
Glycyrrhizic acid
17α-Hydroxyprogesterone
11-Ketotestosterone
Leuprolide acetate
Metformin
Norethindrone
Oxandrolone
Prednisone
Pregnenolone
Progesterone
Salmeterol xinafoate
Spironolactone
Testosterone
17α-Thiomethyl spironolactone
Triptorelin

Calibration interval and onboard stability

Evaluation of calibration interval and onboard stability using the ANDRO assay on the ADVIA Centaur XP system demonstrated calibration interval and onboard stability of 35 days.

Table 4. Evaluation of calibration interval for the ADVIA Centaur ANDRO assay.

	Low Calibrator	High Calibrator	QC Level 2	Serum	Lithium Heparin Plasma
p-value of fit	0.584	0.119	0.574	0.496	0.703
Days of stability	35	35	35	35	35

Table 5. Evaluation of onboard stability for the ADVIA Centaur ANDRO assay.

	Low Calibrator	High Calibrator	QC Level 2	Serum	Lithium Heparin Plasma
p-value of fit	0.489	0.284	0.823	0.607	0.772
Days of stability	35	35	35	35	35

Conclusion

The performance of the ADVIA Centaur ANDRO assay has been evaluated, and the results demonstrate an accurate and precise method for the measurement of androstenedione in human serum and plasma. The assay correlates well with LC-MS/MS.

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*Information about this device is preliminary. Safety and effectiveness for the uses discussed have not been established. The device is under development and not commercially available. Future availability cannot be ensured.

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